The Pending Claims

Prior to entry of the above amendments, Claims 4-7, 9-15, 19, and 23-33 are pending. Claims 23, 4-7, 9-11, 26 and 28 are directed to methods for production of a mutant high alkaline protease; Claims 12-13 and 27 are directed to a method of obtaining an alkalophilic *Bacillus* strain having a reduced extracellular alkaline protease level; Claims 14-15 and 29 are directed to an alkalophilic *Bacillus* strain producing a mutant high alkaline protease; Claim 19 is directed to a detergent composition comprising as an active ingredient a mutant high alkaline protease. Claim 24 is directed to a method of preparing a detergent composition comprising a mutant high alkaline protease as an active ingredient. Claim 25 is directed to a method of processing laundry with the claimed detergent composition. Claims 30-33 are to a method for producing a mutated high alkaline protease free of endogenous extracellular alkaline protease.

The Office Action

The specification is objected to and Claims 4-7, 9-16, 19, and 23-29 and new claims 30-33 are rejected under 35 U.S.C. 112, first paragraph. Claim 23, and thus dependent claims 4-11, and claim 30, and thus dependent claims 31-33 are rejected under 35 U.S.C. § 112, second paragraph. The amendment filed 11-22-94 is objected to under 35 U.S.C. § 132 because it introduces new matter into the specification. The specification is objected to under 35 U.S.C. § 112, first paragraph, and Claim 28 is rejected under 35 U.S.C. § 112, first paragraph, for new matter. Claims 4-7, 9-16, 19, and 23-29 are rejected under 35 U.S.C. § 103 as being unpatentable over Fahnestock *et al.* and Estell *et al.*, in view of TeNijenhuis and Suggs *et al.*

Amendments

Claims 4 and 13 are amended to indicate that the derivatives retain the recited properties of the parent strain. Support for the amendment is found in the specification, for example, at pages 4, 6, 7-9 and 12 and in base Claims 23 and 12.

Claims 14, 23, 26, 29, 30 and 33 are amended to remove the word "substantially." Support for the amendment is found throughout the specification and claims as filed. Claims 19, 24, and 25 are amended to clarify that the detergent composition comprises at least one of the mutant high alkaline proteases prepared according to the methods of the invention. Support for the amendment is found in the claims before amendment and throughout the specification as filed. New Claims 34-37 are added by this amendment and are directed to non-reverting protease-negative alkalophilic strains of *Bacillus*. Support for the claims and claim language is found *inter alia* on pages 4, 6, 7-9 and 12 of the specification, and in the Examples, and in Claims 12, 14, 23, 26-33.

No new matter has been added by any of the amendments and the Examiner is respectfully requested to enter them.

35 U.S.C. § 112, first paragraph

The specification is objected to and Claims 4-7, 9-16, 19, and 23-29 and new claims 30-33 are rejected under 35 U.S.C. 112, first paragraph, as the disclosure is enabling only for claims limited to methods of producing an alkalophilic asporogenic Bacillus novo species PB92 of minimal natural extracellular protease level, transformed with a B. novo PB92 alkaline protease which has been mutated as described in the specification. See M.P.E.P. §§ 706.03(n) and 706.03(z).

Applicants' arguments concerning the mutation of the high alkaline protease gene have been deemed persuasive. Applicants have convincingly demonstrated that the mutation to the protease per se is not the significant part of the application and invention, as stated, for example, at the sentence bridging pages 12-13.

The claims are not properly enabled for the recitation of any "mutant high alkaline protease", and any "alkalophilic Bacillus strain". Applicants have stated that the strain PB92 has been used merely as an example, and that the specification provides enablement for the use of other types of these strains, and for other "mutant high alkaline proteases". Applicants further state that techniques for such are "routine and require no inventive skill or undue experimentation" (pg. 7, response of 9-7-93). This is not deemed persuasive for the reasons of record. Primarily, the specification has not provided pertinent information regarding any other "high alkaline protease" gene, nor any appropriate Bacillus strain that would satisfy the requirements of the invention. This fact is important, as the claims are not commensurate in scope with the specification and its enablement. This information is essential to the function of the claimed invention, and the essential material may not be improperly incorporated into the specification, and does not find support within the teachings of the specification. There is no teaching or reasonable expectation provided that the one skilled in the art would be able to utilize the teachings provided for any other systems/genes, or even that there is a problem with any other source such that the instant invention would be applicable. Absent this knowledge, one skilled in the art is left with an undue amount of experimentation, due to the breadth of the claims, in order to attempt to determine what other Bacilli or proteases would be useful in the instant invention, and then further attempt to find the gene and apply the principles taught herein. Thus, one skilled in the art would in no way be enabled to practice the claimed invention with any such gene or strain other than the enabled Bacillus PB92.

utilize the teachings pr vided for any other systems/genes, r even that there is a pr blem with any ther source such that the instant invention w uld be applicable. Absent this kn wledge, one skilled in the art is left with an undue am unt f experimentation, due to the breadth f the claims, in order to attempt t determine what ther Bacilli r pr teases w uld be useful in the instant invention, and then further attempt to find the gene and apply the principles taught herein. Thus, one skilled in the art would in no way be enabled to practice the claimed invention with any such gene or strain other than the enabled Bacillus PB92.

The rejection to Claim 16 is moot as it was cancelled in a previous amendment. The objection to the specification and rejection to Claims 4-7, 9-15, 23-29 and 30-33 are respectfully traversed in part because it is not relevant which asporogenic and/or alkalophilic *Bacillus* strain is used to practice the method of the invention. The rejection also is traversed in part because, in possession of Applicants' disclosure, one of ordinary skill in the art is taught how to make and use, without undue experimentation, asporogenic and/or alkalophilic *Bacillus* strains and alkaline protease genes in addition to those disclosed in the specification.

The *Bacillus* novo species PB92 strains and the PB92 proteins cited by the Examiner are but examples of alkalophilic *Bacillus* host strains and high alkaline proteases disclosed in the specification. The specification discloses other alkalophilic *Bacillus* strains, such as *B. lentus*, that can be used for protease production (pg. 12 lines 8-21). The specification additionally discloses that the claimed methods find use in the production of mutant serine proteases using protease negative strains of alkalophilic *Bacillus* (pg. 4, lines 32-35; sentence bridging pp. 6-7). Many serine protease genes and proteins are known in the art, are mutatable, and DNA transformation protocols needed for alkalophilic (or asporogenic) *Bacillus* strains have been disclosed (pg. 14, lines 23-25). Alkaline protease genes in addition to the PB92 alkaline protease also are disclosed; for example, *B. lentus* strains are disclosed which produce the mutant high alkaline proteases Esperase® and Savinase® (pgs. 12-18). Other alkalophilic strains and their endogenous proteases also are known in the art and/or obtainable without undue experimentation.

Methods for selecting and characterizing protease negative strains is provided on pg. 17, for example, and methods for evaluating protease activity are provided on pg. 28 line 27 - pg. 29 line 45, for example. Knowledge of the protease gene structure before or after deletion is not essential to practice the instant invention since the deletion is characterized by retention of the non-reverting protease-negative phenotype following stable transformation of a gene encoding an exogenous high alkaline protease of interest. For example, transformation of a

altered protease activity (pg. 28, lines 27 - pg. 29, lines 12-16) or maintains the capacity to degrade substrate (pg. 13, lines 10-13).

One of ordinary skill in the art also would understand that a *Bacillus* protease gene could be deleted by introducing a vector having the 5' and 3' flanking regions but not the coding region of the protease gene. For example, the skilled artisan can easily determine the termini of a test protease gene in a plasmid by using standard restriction enzyme mapping techniques. Once the protease gene termini are determined, a restriction enzyme is chosen that removes the test protease gene sequence from the plasmid, but leaves the termini within the plasmid. Appropriate starting vectors and routine methods for manipulating DNA fragments between plasmids are disclosed pp. 15-16. Applicants urge that these techniques are routine and require no inventive skill or undue experimentation. Once a plasmid is made that comprises the termini of the test protease gene, it can be used to transform a *Bacillus* host (i.e., PB92), resulting in integration and inactivation of the host protease gene (pp. 16-17). The original plasmid comprising the test protease gene can be mutated by digestion with another restriction enzyme and used to transform the mutant *Bacillus* host in order to produce mutant protease (pg. 25-27).

Simply because not all possibly useful *Bacillus* strains and genes encoding extracellular high alkaline proteases are known or have not yet been cloned does not mean that Applicants are limited to what they have exemplified. If that were true, others could with impunity use Applicants' inventive concept simply by substituting another protease gene as the target gene for removal, and substituting a *Bacillus* other than PB92 or PBT110 as the expression host. Applicants have shown that their invention is operable as claimed and therefore are entitled to their generic claims.

Accordingly, Applicants respectfully submit that the specification and claims are enabled and request the objection to the specification and rejection to Claims 4-7, 9-15, 19, 23-29, and 30-33 under 35 U.S.C. 112, first paragraph, be withdrawn.

The specification is not properly enabled for claims to any "derivative thereof" of a Bacillus novo species PB92. Applicants state that passages on page 12 of the specification refer to known "derivatives", and that this would be enabling for the instant invention. The phrase "derivatives thereto", however, encompasses predetermined and random mutants of the strain, and progeny of the strain that may or may not contain that gene for the "mutant high alkaline protease" and/or a revertant strain with the indigenous gene. The specification does not properly teach nor describe to one skilled in the art these "derivatives", what specifically they entail, nor how to obtain and/or use such. Mere reference to other teachings, when this is a matter of essential material, without an instant and specific teaching as to how these would be applicable, is not sufficient. Thus, this results in undue experimentation for one skilled in the art to attempt to produce such without pr per guidance from the specification.

The rejection is avoided by amendment of claims 4 and 13 to recite that the derivatives and/or progeny are non-reverting and contain a mutant high alkaline protease gene. Examples which employ *Bacillus* novo species PB92 and how to make and use derivatives using standard techniques known in the art (pg. 10, line 11 - pg. 12, line 7) are provided in the specification. The specification references other derivatives of *Bacillus* novo species PB92 (pg. 12, lines 8-21) which were obtained using procedures known to those in the art and described in the specification (pg. 10, line 11 - pg. 12, line 7). These are examples of derivatives that are in addition to and not essential material to the working examples and disclosure of the instant application. Indeed, predetermined mutants of a species can be made, as correctly noted by the Examiner, as taught and enabled by the specification. For example, "derivatives" according to the specification are strains derived from PB92 in which a desired property is introduced (*e.g.* secretion improvement) or an undesired property is deleted (*e.g.* sporulation, see Example 2: PBT-110). The exact construction/selection conditions for obtaining a derivative depends in part on the strain used and the conditions in which this strain is used and can be determined empirically by one of ordinary skill in the art. The derivative strains are obtained by standard microbiological molecular/biological processes which are familiar to one of ordinary skill in the art.

In view of the above remarks, the Examiner is respectfully requested to withdraw the rejection.

New claim 29 is not properly enabled by the teachings of the specification for the host strain to be "substantially incapable of reversion". The specification, at page 7, lines 16-17, teach that the invention is (preferably) a "non-reverting mutation". This clearly conflicts with the phrase "substantially incapable". Thus, one skilled in the art has not been taught by the specification, nor is enabled for, the production of such strains that are only "substantially incapable" of reversion. Similarly, since this is the case, and the gene encoding the high alkaline protease has been deleted, then it follows that there should be no "indigenous" alkaline protease product. Thus, it also follows that such a strain could not be "substantially free" of an indigenous alkaline protease, but instead, only completely free. The teachings of the specification do not provide support for such a strain being "substantially free", nor does the specification teach one skilled in the art how to produce such a strain.

The rejection is avoided in view of the amendment removing the word "substantially." Accordingly, the Examiner is respectfully requested to withdraw the rejection.

It should be noted that applicants have pointed out, at page 17 of the response filed 11-22-94, that the Examiner has erred in rejecting claim number 9 under 35 USC 112, 1st paragraph. This is not deemed persuasive. Although the claim is specific with regard to the source of the protease gene, it has not overc me all the deficiencies specified above for the

claim(s) from which it is dependent (ultimately claim 23). This can be easily seen, f r example, if the limitati ns f claim 9 were t be included with the limitations f the claims it depends up n in ne singular claim. Thus, it is specific (clearly enabled) for ne aspect, but not all, and is still pr perly rejected.

The rejection is avoided in view of the amendments filed July 11, 1995, the instant amendments which remove the word "substantially" from base Claim 23, and the remarks that follow. The Examiner rejected Claim 23 for not enabling all alkalophilic *Bacilli* and all high alkaline protease genes. Claim 7 limits the expression cassette of base Claim 23 to a plasmid. Claim 9 limits Claim 7 to comprise a high alkaline protease obtained from *Bacillus* novo species PB92. Applicants respectfully submit that in possession of the PB92 high alkaline protease gene according to Claim 9, one of ordinary skill in the art can easily practice the method according to Claim 9 by (1) transforming a test alkalophilic *Bacillus* strain that is protease-negative for endogenous extracellular protease (see, e.g., specification pg. 17, describing selection and evaluation of protease-negative strains of *Bacilli*) with a plasmid comprising the mutant PB92 high alkaline protease gene, and (2) screening for stable transformants that are incapable of reversion to a protease-positive phenotype and efficiently produce the desired mutant PB92 protease (see, e.g., specification page 13, lines 10-13, and pg. 28, line 27 through page 29, line 45, describing evaluation of protease activity). Protease-negative alkalophilic *Bacillus* test strains incapable of reversion will necessarily comprise a deletion of the gene for endogenous extracellular protease required by base Claim 23. Thus in possession of Applicants' disclosure and that known in the art, the method of Claim 9 is a matter of routine screening and selection. In view of the above remarks and amendments, the Examiner is respectfully requested to withdraw the rejection.

35 U.S.C. § 112, second paragraph.

Claim 23, and thus dependent claims 4-11, and claim 30, and thus dependence claims 31-33 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which appellant regards as the invention.

Claims 23 and 30 are indefinite, as the recitation of the phrase "substantially free of indigenous" protease at line 2, while line 5 conflicts with the phrase "no detectable indigenous" protease. The relationship of "substantially free" and "no detectable" would not appear to be the same.

The rejection to Claims 23, 4-11, and 30-33 are avoided by the amendments to the claims to delete the word "substantially." Accordingly, the Examiner is respectfully requested to withdraw the rejection.

35 U.S.C. § 132

The amendment filed 11-22-94 is bjected to under 35 U.S.C. § 132 because it introduces new matter into the specificati n. 35 U.S.C. § 132 states that no amendment shall introduce new matter into the disclosure of the invention. The added material which is not supported by the original disclosure is as follows:

The phrases "minimal indigenous extracellular protease level", and "a specifically-mutated Bacillus novo PB92 alkaline protease" (underlining added for emphasis) in claim 28.

Applicants, at page 8 of the response, have stated that since the Examiner "indicated as enabled" these phrases, that a claim quoting these phrases seemed proper. This is not. These phrases are merely quotes from the Office Action, in the Examiner's shorthand manner of expressing the enablement of the specification. Obviously, the specification deals with very low levels of expression product, but does not exactly mention "minimal indigenous" protease. Although the two might be grammatically interchangeable, this was not a suggestion or invitation to use this in the claims. This also applies to the phrase "specifically-mutated", which was a shorthand way of avoiding the listing of each mutation used or available to applicant.

Applicant is required to cancel the new matter in the response to this Office Action.

The rejection is avoided in part by the amendment of Claim 28 to recite "reduced" instead of "minimal;" this term and the term "indigenous" in reference to the alkaline protease gene is used in Claim 12 as filed. The rejection is traversed in part because the phrase "specifically-mutated" is found in the specification and carries with it the definition as used in the art (see, e.g., specification pg. 10, line 5). How the specification describes the invention is not subject to limitation by specific words or phraseology. M.P.E.P. § 608.01(o).

Accordingly, the specification is clear in its description of the invention, including the those claim limitations not expressly quoted by the specification, and the Examiner is respectfully requested to withdraw the rejection.

35 U.S.C. § 112, first paragraph

The specification is objected to under 35 U.S.C. § 112, first paragraph, as the specification, as originally filed, does not provide support for the invention as is now claimed. This is deemed necessary for the reasons recited immediately above.

Claim 28 is rejected under 35 U.S.C. § 112, first paragraph, for the reasons set forth immediately above in the objection to the specification.

The objection to the specification and the rejection of Claim 28 should be withdrawn for the reasons stated above.

35 U.S.C. § 103

Claims 4-7, 9-16, 19, 23-29 and new claims 30-33 are rejected under 35 U.S.C. § 103 as being unpatentable over Fahnest ck *et al.* and Estell *et al.*, in view of TeNijenhuis and Suggs *et al.* The references and rejection are herein incorporated as cited in a previ us Office Action.

Applicants' arguments filed in response to this rejection have been fully considered but they are not deemed to be persuasive. Arguments submitted 11-22-94 do not substantially differ from those already presented previously, and thus are not deemed persuasive for the reasons of record. Regarding claims 30-33, they are included for the same reasons as the previously rejected claims. Also, it is noted that claims 30-33 read upon a method of isolating an enzyme from a culture broth, which was well known in the art at the time the invention was made. The limitations of the host cell from which the enzyme is isolated does not render the steps of the method (only recited as isolating, and nothing else) patentable *per se*.

Claim 16 has been canceled in a previous response, and thus the rejection to this claim is moot. It also is noted that the Examiner refers only to Applicants' arguments provided up to and including their response of 11/22/94, even though a full response with amendments and new arguments was filed 7/10/95. The Examiner is reminded that he is obliged to give due consideration to each amendment and argument raised by an applicants' response. Applicants' response of 07/10/95 is hereby incorporated by reference.

The rejection to independent Claims 12, 23, 26, 27, 28, 29 and 30 (and the rejected dependent Claims 4-7, 9-15, 24-25, 31-33) is respectfully traversed because the cited references would not have made obvious the claimed invention at the time the invention was made.

As reviewed in the "SUMMARY OF THE INVENTION" (pg. 4), Applicants' invention relates to the production of mutant high alkaline protease, free of the wild-type endogenous protease, preferably in an alkalophilic strain of *Bacillus*. The invention provides surprising and unexpected advantages over the cited art of Estell *et al.*, Fahnestock *et al.*, TeNijenhuis *et al.* and Suggs *et al.*, in that efficient production of exogenous mutant protease is obtained in a *Bacillus* host strain which is unable to produce a wild-type endogenous protease because it is incapable of reversion back to wild-type. This is accomplished by deleting the endogenous protease gene through replacement of the genes' coding sequence with a gene of interest, or any other method which produces endogenous protease sequences that are too short for recombination with sequences homologous to the host strain or to the exogenous mutant high alkaline protease gene. In this way, reversion of the protease-negative phenotype and the exogenous mutant protease genotype is prevented. The efficiency in production is improved *inter alia* by preventing (1) reversion and expression of the endogenous wild-type protease gene in the *Bacillus* host strain and (2) reversion of the

exogenous mutant protease gene, where reversion contaminates the desired product and reduces metabolic efficiency of production.

The rejected method (Claims 4-7, 9-15, 19, 23-28 and 30-33) and composition claims (Claims 14-15, 19, and 29) of the invention encompass the above embodiments and would not have been obvious over the cited references at the time the invention was made. None of the references teach or suggest the method of Claims 23, 4-7, 9-11, 26 and 28, which methods require production of a mutant high alkaline protease free of indigenous extracellular high alkaline protease in an alkalophilic *Bacillus* host strain (Claims 23, 4-7, 9-11 and 28), or reduced levels of indigenous extracellular protease in an asporogenic *Bacillus* host strain (Claim 26). The references also fail to teach or suggest methods for Claims 12-13 and 27, which are directed to a method of obtaining an alkalophilic *Bacillus* strain having no detectable extracellular high alkaline protease. Thus, the method of Claim 24 (preparing a detergent composition comprising a mutant high alkaline protease as an active ingredient) and Claim 25 (processing laundry with the claimed detergent composition) can not be obvious. Likewise, the method Claims 30-33 for producing a mutated high alkaline protease free of endogenous extracellular high alkaline protease in *Bacillus* novo species PB92 is not taught or suggested by the cited references.

The references would not have made obvious the composition of Claims 14-15 and 29 to an alkalophilic *Bacillus* strain producing a mutant high alkaline protease free of endogenous extracellular alkaline protease incapable of reversion or Claim 19, which is directed to a detergent composition comprising as an active ingredient a mutant high alkaline protease produced according to a method of the invention, because the mutant high alkaline protease used in the composition was not available prior to Applicants' invention. /wrong!!

The specific deficiencies of the combination of the cited references are discussed below.

Primary references of Fahnestock *et al.* and Estell *et al.*

Estell *et al.* and Fahnestock *et al.* do not teach or suggest construction or use of alkalophilic and/or asporogenic *Bacillus* strains that are non-reverting for production of exogenous protease free of endogenous alkaline protease, and certainly not production of exogenous extracellular high alkaline protease free of endogenous alkaline proteases). Nor do Fahnestock *et al.* and Estell *et al.* provide reasonable expectation that such strains or high

alkaline protease could be produced free of indigenous protease, nor do they disclose the unexpected results obtained by Applicants. Claims 4, 6-7, 9-16, 23-25, 27, 29, 30-31 and 33 of the present invention require a *Bacillus* strain that is alkalophilic, and Claims 5, 28 and 32 require a *Bacillus* strain that is both alkalophilic and asporogenic. Fahnestock *et al.* and Estell *et al.* are exemplified by the use of *B. subtilis*, which strain is not alkalophilic. That alkalophilic *Bacilli* were simply known to exist in the art (*e.g.* TeNijenhuis) is of no consequence because the primary references specifically teach and suggest the use of their methods with non-alkalophilic *Bacilli*; there is no suggestion or incentive to attempt the methods of Fahnestock *et al.* and Estell *et al.* with any alkalophilic *Bacilli* and certainly no expectation that their methods might be applicable in any *Bacillus* strain other than those that are non-alkalophilic. Indeed, undermining the actual teachings of Fahnestock *et al.* and Estell *et al.*, and thus making the Examiner's extrapolation from non-alkalophilic to alkalophilic *Bacillus* one requiring a leap of faith, were problems known in the art when *Bacilli* were used to produce proteases (see. e.g., specification pg. 1, line 31 - pg. 2, lines 1-19), including the fact that special methods of transformation are required for alkalophilic *Bacilli* (see. e.g., specification pg. 14, lines 23-25). Additionally, neither Estell *et al.* nor Fahnestock *et al.* teaches or suggest the production of any high alkaline protease much less mutant high alkaline protease in the absence of expression of indigenous protease.

Claim 26 of the instant invention requires a *Bacillus* strain that is asporogenic for production of extracellular high alkaline protease. Claims 5 and 28 require a *Bacillus* strain that is both asporogenic and alkalophilic for production of mutant extracellular high alkaline protease. In contrast, Fahnestock *et al.* disclose an asporogenic *B. subtilis* strain, which is not alkalophilic, expressing chloramphenicol resistance from a CAT gene insert. CAT is not a protease, much less a high alkaline protease. Fahnestock *et al.* do not teach production of any protease in asporogenic strains of *Bacilli* nor is there a motivation to do so. Estell *et al.* expressly teach away from using asporogenic strains of *Bacilli* for the production of any recombinant protease of interest (see, e.g., Applicants' specification pg. 2, lines 5-16, page 3, lines 24-31). Based upon Fahnestock *et al.*, there would have been no reasonable expectation of success given the express teaching by Estell *et al.* that asporogenic *Bacillus* strains were not suitable for such expression. The only motivation or reasonable expectation is provided by

Applicants' disclosure, which is not available as a blueprint to reconstruct a *prima facie* case in hindsight. Indeed, Applicants demonstrated that asporogenic strains of *Bacillus* are surprising good producers of extracellular high alkaline protease, which is unexpected with respect to the cited art (see, e.g., Applicants' specification page 2, lines 5-16, page 3, lines 24-31). This is because there was no agreement in the art as to whether asporogenic strains of *Bacillus* could be used for production of any recombinant protein. Therefore, claims so limited could not have been obvious (Claims 5, 26, 28).

Fahnestock *et al.* and Estell *et al.* do not disclose or suggest a method of producing extracellular high alkaline protease free of endogenous alkaline protease incapable of reversion or its production in a *Bacillus* host strain having an extracellular protease-negative phenotype that is incapable of reversion. Fahnestock *et al.* and Estell *et al.* teach methods for obtaining *Bacillus* strains which contain an indigenous extracellular non-high alkaline protease which is alleged to be inactivated by (1) insertion mutagenesis using an antibiotic marker gene[1] or (2) partial deletion of the coding region by chemical mutagenesis[2], respectively. Both methods result in a *Bacillus* strain that is cable of reversion from a protease-negative strain back to the wild-type protease-positive strain. Both methods result in strains that are capable of reversion/recombination with exogenously introduced extracellular alkaline protease gene. This is evidenced by the fact that the *Bacillus* strains of Estell *et al.* and Fahnestock *et al.* are reported to express a protein encoded by an exogenously introduced gene in the absence of detectable levels of an endogenous extracellular neutral protease, but only reduced levels of

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[1] Fahnestock *et al.* teaches a method of insertion mutagenesis where the inactivated protease gene is integrated into the chromosome by a "two-step insertion excision process" which occurs simultaneously in the same irreversible reaction: the so called "first step" results in tandem duplication in which one of two copies of the protease gene is unaltered; the so called "second step" resolves the duplication event in which the altered (CAT insertion mutant) or unaltered (indigenous) protease gene is retained (see Fahnestock *et al.*, column 4, lines 17-35, and Figure 4). The outcome of Fahnestock *et al.*'s method is either (1) an inactive indigenous protease gene (CAT insertion) with substantial tracks of flanking coding region present that are prone for reversion back to wild-type and recombination with an exogenous gene encoding a desired mutant protease, or (2) an intact active indigenous protease gene.

[2] The chemical mutagenesis method of Estell *et al.* leaves substantial tracks of coding region and other portions of the gene encoding the target endogenous protease. Thus, both the strain and any exogenous genetic material with regions of homology are capable of reversion with any *Bacillus* strain produced according to the chemical mutagenesis method of Estell *et al.*.

endogenous extracellular alkaline protease. Importantly, the authors reported that the reduced expression of endogenous alkaline protease was due to the presence of indigenous extracellular alkaline protease genes other than the mutated target protease gene. This is in contrast to Applicants' invention in that Applicants demonstrated the necessity of constructing/employing *Bacillus* host strains having an extracellular protease-negative phenotype incapable of reversion by recombination with endogenous, and particularly exogenous extracellular alkaline protease genes of interest. This aspect of Applicants' inventive concept permits stable transformation and production of mutant extracellular alkaline protease free of endogenous extracellular alkaline protease. Accordingly, the methods and strains of Estell *et al.* and Fahnestock *et al.* are flawed and the only suggestion in the references to improve their methods and strains teaches away from the invention claimed by Applicants.

Additionally, neither Estell *et al.* or Fahnestock *et al.* would have provided the motivation to delete the a sufficient amount of endogenous extracellular protease gene coding region to prevent reversion and production of exogenous alkaline protease free of endogenous extracellular alkaline protease for two basic reasons. First, Estell *et al.* and Fahnestock *et al.* do not provide the motivation to delete the entire coding region of an indigenous extracellular protease gene because one of ordinary skill in the art would not have expected such a step to provide any different results than those already obtained by their methods. Estell *et al.* and Fahnestock *et al.* suggest that their methods effectively destroyed the target protease gene's activity by either insertion (*e.g.*, CAT gene insertion) or partial deletion (*e.g.*, chemical mutagenesis). Thus one of ordinary skill in the art would not have been motivated to delete the entire protease gene or its coding region because such a step would have been expected to be redundant and not expected to provide any improved qualities for producing any protein in the resultant *Bacillus* strain.

Second, Estell *et al.* and Fahnestock *et al.* both teach away from further manipulation of the target endogenous protease gene. For example, Fahnestock *et al.* teaches that to further reduce extracellular protease activity, other endogenous extracellular protease genes are to be disrupted by their method of insertional mutation (Fahnestock *et al.* col. 4). Fahnestock's method for insertion mutagenesis does not prevent reversion. Similarly, Estell's chemical

mutagenesis method does not prevent reversion. Thus, neither reference provides a method for constructing non-reverting extracellular protease-negative *Bacillus* strains producing exogenous extracellular protease of any type. There is no mention or suggestion in either Estell *et al.* or Fahnestock *et al.* to obtain such strains incapable of reversion nor why it would be advantageous. Accordingly, one of ordinary skill in the art would not have been motivated to veer from the teachings of Fahnestock *et al.* and Estell *et al.* and thus would not have been motivated to attempt deletion of a sufficient portion of the coding region of a target indigenous extracellular alkaline protease gene to prevent reversion of both the protease-negative phenotype and production of extracellular alkaline protease free of indigenous extracellular alkaline protease.

Secondary references of TeNijenhuis *et al.* and Suggs *et al.*

A generic method for cloning human cDNA based on partial amino acid sequence data (Suggs *et al.*) and the fermentation and partial purification of a bacterial protease enzyme (TeNijenhuis *et al.*) adds nothing to the teachings of Estell *et al.* and Fahnestock *et al.* that would have rendered obvious Applicants' inventive concept for reasons already discussed above.

Indeed, the claimed invention provides surprising and unexpected advantages over the cited art of Estell *et al.*, Fahnestock *et al.*, TeNijenhuis *et al.* and Suggs *et al.*, in that efficient production of exogenous mutant protease is obtained in a *Bacillus* host strain which is unable to produce a wild-type endogenous protease because it is incapable of reversion back to wild-type. The efficiency in production is surprising and unexpected, for example, because in the absence of reversion of the host strain or the exogenous mutant protease, the stability of plasmids (comprising sequences homologous between exogenous mutant protease and the wild-type genomic sequence capable of reversion) is increased (see, e.g., specification pg. 2, lines 25-29 and pg. 7, lines 30-38), and the metabolic efficiency is increased (see. e.g., specification pg. 2, lines 32-34, pg. 12, lines 22-28). The efficiency in production also is surprising and unexpected, for example, because the industrial *Bacillus* strain in which mutant protease gene is integrated from which the wild-type gene is deleted, gives a production level which is similar to strains whereby the gene is multi-copy situated (see, e.g., specification pg. 9, lines 35, through page 10, line 2, and pg. 12, lines 1-17). Thus, even if the Examiner's

combination of references were on-point, the rejection is in error because of the surprising and unexpected advantages demonstrated by Applicants claimed invention over the cited art.

Accordingly, Applicants maintain that the claimed invention is patentable over the cited references and the Examiner is respectfully requested to withdraw the rejection.

CONCLUSION

In view of the above amendment and remarks, it is submitted that this application is now ready for allowance. Early notice to that effect is solicited. If in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned at (415) 328-4400.

Respectfully submitted,

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